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EXAMINER	
GOLDBERG, JEANINE ANNE	

ART UNIT	PAPER NUMBER
1634	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/085,774	SYLVAN, ANNA	
	Examiner	Art Unit	
	Jeanine A Goldberg	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 October 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,4-15,17 and 20-25* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,4-15,17 and 20-25* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. This action is in response to the papers filed October 1, 2004. Currently, claims 1, 4-15, 17, 20-25 are pending.

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on October 1, 2004 has been entered.

3. Any objections and rejections not reiterated below are hereby withdrawn in view of the amendments to the claims to required adding non-chain terminating nucleotides and applicant's arguments.

New Grounds of Rejection Necessitated by Amendment

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

4. Claims 1, 7, 17 are rejected under 35 U.S.C. 102(e) as being anticipated by Shuber et al. (US Pat. 6,566,101, May 20, 2003).

Shuber et al. (herein referred to as Shuber) teaches a method for selective nucleic acid sequence detection in single base primer extension reactions of high sensitivity. The methods are useful in detecting small amounts of mutant nucleic acid in a heterogeneous biological sample (abstract). Shuber teaches that samples may be pooled to determine the number of a nucleic acid in a sample (col. 14, lines 60-68). Shuber teaches that the primer extension to identify a single nucleotide polymorphic variant as the present may be performed on combined samples (col. 33, lines 10-15)(limitations of Claim 1). Shuber teaches labeled dNTPs preferably comprise a detection moiety which facilitates detection of the extended primer (col. 5, lines 10-15)(limitations of Claim 1). Shuber teaches to detecting single nucleotide polymorphisms (SNPs) a primer is designed so that the 3' end of the hybridized primer is immediately upstream of the position that is complementary to the nucleotide position being assayed (col. 6, lines 27-32)(limitations of Claim 1). The primers are preferably extended with a nucleotide labeled with an impedence bead and the number of impedence beads is counted and the number of labeled primers are then determined from the number of impedence beads (col. 7, lines 27-35). Shuber teaches that genomic nucleic acid samples are isolated from a biological sample (col. 7, lines 4-5). Amplification of the specific regions present in the nucleic acids my be amplified by PCR to provide the advantage of increasing the concentration of specific nucleic acid sequences within the target nucleic acid sequence population (col. 7, lines 5-10). The nucleic acids may be bound to a solid phase support which allows the simultaneous processing and screening of a large number of samples (col. 7, lines 13-17)(limitations

of Claim 7). Schuber teaches that the method compares the number of molecules of two nucleic acids that are expected to be present in the sample in equal numbers in normal cells. The comparison is between an amount of a genomic polynucleotide that is known or suspected not to be mutated in cells of the sample and an amount of wild-type genomic polynucleotide segment suspected of being mutated in a subpoplation of cells in the sample (i.e. frequency)(limitations of Claim 1). Therefore, since Shuber teaches every limitation of the instant claims, Shuber anticipates the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. Claims 4-7, 20-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nyren-2 (WO 98/28440, published July 2, 1998) or in view of Shuber et al. (US Pat. 6,566,101, May 20, 2003).

Nyren-2 teaches a method of sequencing DNA based on the detection of the release of pyrophosphate. In example 2, Nryren-2 teaches pyrosequencing on a PCR product. The biotinylated PCR products were immobilized onto a Dynabead (page 38)(limitations of Claim 7, 23). The primer was hybridized to the template and incubated with polymerase (page 38). The sequence procedure was carried out as a stepwise elongation of the primer-strand upon sequential addition of the different

deoxynueoside tripohsphates and simultaneous degradation of the nucleotides by apyrase (col. 38)(limitations of Claim 6, 22). Released Ppi due to nucleotide incorporated was detected (page 39)(limitations of Claim 4, 20). Thus, the base was identified at a target position. Nyren-2 teaches identifying a base at a target position in a sample DNA sequence wherein an extension primer, which hybridizes to the sample DNA immediately adjacent to the target position is provided and the sample DNA and extension primer are subjected to polymerase reaction in the presence of a deoxynucleotide where the deoxynucleotide will only become incorporated and release pyrophosphate (PPI) if it is complementary to the base in the target position. Any release of Ppi being detectable enzymically. Nyren-2 teaches including a nucleotide degrading enzyme during the polymerase reaction step, such that unincorporated nucleotides are degraded (page 3, para 2). Nyren-2 teaches that luciferin-luciferase reactions to detect the release of Ppi are well known in the art (page 7)(limitations of Claim 24-25). Nyren-2 specifically teaches that apyrase is a nucleotide-degrading enzyme (page 4). Nyren-2 teaches that including a nucleotide-degrading enzyme allows the sequencing procedure to proceed without washing the template between successive nucleotide additions. Additionally, since washing steps are avoided, it is not necessary to add new enzymes (page 5).

Nyren does not specifically teach pooling the nucleic acid molecules into a sample and determining the frequency of an allele in a population.

However, Shuber et al. (herein referred to as Shuber) teaches a method for selective nucleic acid sequence detection in single base primer extension reactions of

high sensitivity. The methods are useful in detecting small amounts of mutant nucleic acid in a heterogeneous biological sample (abstract). Shuber teaches that samples may be pooled to determine the number of a nucleic acid in a sample (col. 14, lines 60-68). Shuber teaches that the primer extension to identify a single nucleotide polymorphic variant as the present may be performed on combined samples (col. 33, lines 10-15)(limitations of Claim 1). Shuber teaches labeled dNTPs preferably comprise a detection moiety which facilitates detection of the extended primer (col. 5, lines 10-15)(limitations of Claim 1). Shuber teaches to detecting single nucleotide polymorphisms (SNPs) a primer is designed so that the 3' end of the hybridized primer is immediately upstream of the position that is complementary to the nucleotide position being assayed (col. 6, lines 27-32)(limitations of Claim 1). The primers are preferably extended with a nucleotide labeled with an impedence bead and the number of impedence beads is counted and the number of labeled primers are then determined from the number of impedence beads (col. 7, lines 27-35). Shuber teaches that genomic nucleic acid samples are isolated from a biological sample (col. 7, lines 4-5). Amplification of the specific regions present in the nucleic acids my be amplified by PCR to provide the advantage of increasing the concentration of specific nucleic acid sequences within the target nucleic acid sequence population (col. 7, lines 5-10). The nucleic acids may be bound to a solid phase support which allows the simultaneous processing and screening of a large number of samples (col. 7, lines 13-17)(limitations of Claim 7). Schuber teaches that the method compares the number of molecules of two nucleic acids that are expected to be present in the sample in equal numbers in

normal cells. The comparison is between an amount of a genomic polynucleotide that is known or suspected not to be mutated in cells of the sample and an amount of wild-type genomic polynucleotide segment suspected of being mutated in a subpoplation of cells in the sample (i.e. frequency)(limitations of Claim 1).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the method of Nyren-2 which detect alleles by the primer extension method which detect the release of pyrophoshate with the pooling primer extension method of Shuber. The ordinary artisan would have been motivated to have pooled a population for the expected benefits taught by Shuber. Shuber specifically teaches that pooled samples are useful to screen large numbers of individuals, to identify genomic features such as mutations or SNPs indicative or associated with a disease. Therefore, applying the primer extension method which detects Ppi released with the pooling primer extension method of Shuber would have been obvious to also for more rapid high throughput analysis of samples.

Response to Arguments

The response traverses the rejection. The response asserts there would have been no motivation to combine Nyren-2 with Lapidus (response filed August 30, 2004, page 9). This argument has been reviewed as it may pertain to the newly added rejection of Nyren-2 in view of Shuber. The response asserts that Nyren-2 only teaches a method of sequencing DNA and not for any purpose other than sequencing and sequencing related applications such as the detection of single base changes. This argument has been reviewed but is not convincing because Nyren specifically teaches

placing an extension primer with a sequence immediately 5' of the target position (page 15). Moreover, Nyren-2 teaches the method of the invention may also be used for real-time detection of known single-base changes (page 27). Thus, Nyren-2 does specifically teach applications such as detection of single base changes. Additionally, the response points to page 18, lines 13-27 of Nyren-2 which is specifically directed to detecting carriers for disease by detecting single base changes (see response from August 30, 2004, page 9-10).

The response asserts that the mere use of pooled samples does not provide motivation to use pooled sample in the method of Nyren-2. This argument has been thoroughly reviewed, but is not found persuasive because the ordinary artisan would have been motivated to have pooled samples to screen large number of individuals rapidly.

The response further asserts that that "one would have expected that the method of Nyren-2 would be suitable only for a homozygous sample or a heterozygous sample from a single individual" (response page 10 of August 30, 2004). This argument has been thoroughly reviewed, but is not found persuasive because whether the sample is a heterozygous sample from a single individual or a heterozygous sample from multiple individuals will allow detection of the quantity of particular signals in the aliquots (see page 18, lines 13-27 of Nyren-2). The response asserts that one would not have expected the use of Nyren-2 in a pooling method, however, fails to provide any evidence or supporting analysis for the argument. MPEP 716.01(c) makes clear that "The arguments of counsel cannot take the place of evidence in the record. In re

Schulze , 346 F.2d 600, 602, 145 USPQ 716, 718 (CCPA 1965). Examples of attorney statements which are not evidence and which must be supported by an appropriate affidavit or declaration include statements regarding unexpected results, commercial success, solution of a long - felt need, inoperability of the prior art, invention before the date of the reference, and allegations that the author(s) of the prior art derived the disclosed subject matter from the applicant." Here, the statements regarding the unexpected results must be supported by evidence, not argument.

Thus for the reasons above and those already of record, the rejection is maintained.

6. Claims 8-11, 14-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Shuber et al. (US Pat. 6,566,101, May 20, 2003) in view of Breen et al. (BioTechniques, Vol. 28, No. 3, pages 464-470, March 2000).

Shuber et al. (herein referred to as Shuber) teaches a method for selective nucleic acid sequence detection in single base primer extension reactions of high sensitivity. The methods are useful in detecting small amounts of mutant nucleic acid in a heterogeneous biological sample (abstract). Shuber teaches that samples may be pooled to determine the number of a nucleic acid in a sample (col. 14, lines 60-68). Shuber teaches that the primer extension to identify a single nucleotide polymorphic variant as the present may be performed on combined samples (col. 33, lines 10-15)(limitations of Claim 1). Shuber teaches labeled dNTPs preferably comprise a detection moiety which facilitates detection of the extended primer (col. 5, lines 10-

15)(limitations of Claim 1). Shuber teaches to detecting single nucleotide polymorphisms (SNPs) a primer is designed so that the 3' end of the hybridized primer is immediately upstream of the position that is complementary to the nucleotide position being assayed (col. 6, lines 27-32)(limitations of Claim 1). The primers are preferably extended with a nucleotide labeled with an impedence bead and the number of impedence beads is counted and the number of labeled primers are then determined from the number of impedence beads (col. 7, lines 27-35). Shuber teaches that genomic nucleic acid samples are isolated from a biological sample (col. 7, lines 4-5). Amplification of the specific regions present in the nucleic acids my be amplified by PCR to provide the advantage of increasing the concentration of specific nucleic acid sequences within the target nucleic acid sequence population (col. 7, lines 5-10). The nucleic acids may be bound to a solid phase support which allows the simultaneous processing and screening of a large number of samples (col. 7, lines 13-17)(limitations of Claim 7). Schuber teaches that the method compares the number of molecules of two nucleic acids that are expected to be present in the sample in equal numbers in normal cells. The comparison is between an amount of a genomic polynucleotide that is known or suspected not to be mutated in cells of the sample and an amount of wild-type genomic polynucleotide segment suspected of being mutated in a subpoplation of cells in the sample (i.e. frequency)(limitations of Claim 1).

Shuber does not specifically teach determining the amount or concentration of nucleic acids in each sample prior to pooling.

However, Breen et al. (herein referred to as Breen) teaches methods of pool construction and methods of testing the sensitivity of the DNA pooling method. Breen teaches concentrations of samples may be estimated by fluorimetry. Moreover, Breen teaches testing the accuracy of the pooling protocol by comparing the frequencies derived from individual genotyping with tests using spiking where additional alleles were introduced into DNA pools (page 464, col. 3). Breen teaches dividing the pools into aliquots and the amount of DNA from a homozygote that was equivalent to one, two, five and ten alleles was added to the aliquots. PCR (primer-extension reaction) was carried out on the pools. Moreover, additional calibration pools were constructed and DNA from different homozygotes was mixed together in different ratios (0:100, 20:80, 40:60, etc)(page 464, col. 3). Breen teaches analyzing SNP in the DRD2 gene and the COLIA1 gene (page 464, col. 3). Figure 2 illustrates a calibration curve to correct for the distorted allele frequencies derived from pooling (page 466). Breen teaches that the results of the Taqman assay to determine genotypes was highly consistent and reproducible (page 469, col. 2). The Taqman assay uses both primers and probes (primer extension reaction). Breen teaches using allele specific fluorescent probes to determining the number of copies of the two alleles. Breen illustrates the difference between the estimate and the results of the differing levels of probes was significant, with a p value equivalent to $P<0.0001$ (page 469, col. 3). Thus, Breen teaches that copies of the two alleles may be determined using fluorescent probes, i.e. the total of the two alleles provides an accurate indication of the concentration of the original sample. While Breen specifically teaches using the same concentration of each DNA

sample, Breen does not specifically teach determining the concentration of the nucleic acid by a primer extension reaction prior to pooling.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the method of Shuber with the specific teachings of Breen that concentrations of samples should be ensured. It would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the method of Shuber to ensure that the problems enumerated by Breen were considered. The ordinary artisan would have been motivated to have detected concentrations of each DNA sample using a fluorimeter with a method using allele-specific fluorescent probes as taught by Breen. Breen teaches "it seems the only limiting factor on accuracy in this system is the variation introduced when the pools are constructed and the DNA concentration measurements made." (page 470, col. 1). To obtain accurate information regarding the true frequencies of a pooled population, it is essential that each member of the population is represented in equal concentrations within the sample. For example if the population is comprised of two homozygote individuals, aa and AA, and the pool is designed to contain 10 µg of DNA from the individual with "aa" and 90 µg of DNA from the individual with "AA", the estimated frequency of the alleles would be 0.1 for "a" and 0.9 for "A". This would not accurately reflect the true population frequency. In contrast, a pool designed to contain 50 µg of DNA from the individual with "aa" and 50 µg of DNA from the individual with "AA", the estimated frequency of the alleles would be 0.5 for "a" and 0.5 for "A", a true estimate of the frequencies of the two alleles in the population. Therefore, in order to obtain a true

estimate of the frequency of alleles in a population, adjusting the amount or concentration of a nucleic acid in a sample is essential. It would have been obvious to the ordinary artisan to dilute or increase nucleic acid concentrations to ensure equal concentrations within each of the samples prior to pooling. Thus, because Breen illustrates the accuracy of the allele-specific fluorescent probe system in determining the copy numbers of various alleles in a particular sample, the concentration (the total of all possible alleles in the sample) may be accurately determined. Since the ordinary artisan performing the method of Breen for determining the allele frequencies in a pooled DNA sample using a 5' fluorescently labeled primer teaches the necessity of having equal concentrations of each sample, the ordinary artisan would recognize that a method of determining concentrations of samples using a Taqman assay would be an alternative means of obtaining information regarding concentration of a sample. Breen teaches that the results of the Taqman assay to determine genotypes was highly consistent and reproducible (page 469, col. 2).

With respect to Claim 14-15, Breen teaches DNA from different homozygotes was mixed together in different ratios (as seen in Figure 2). Figure 2 illustrates pooling of samples at different concentrations to determine the relative concentration in each reference sample, thereby generating a calibration cure to correct for the distorted allele frequencies derived from pooling analysis.

Therefore, using the Taqman assay would be an equivalent means of determining the concentration of a particular sample and the ordinary artisan would have been motivated to have used the assay for the benefit of using a highly consistent

and reproducible assay for determining the concentration of nucleic acids present in a sample.

Response to Arguments

The response traverses the rejection. The response asserts a Declaration under 37 C.F.R 1.132 of inventor Anna Sylvan evidencing a date of invention prior to the publication date of Breen has been provided. The declaration filed on August 26, 2004 under 37 CFR 1.131 has been considered but is ineffective to overcome the Breen reference.

The evidence submitted is insufficient to establish a reduction to practice of the invention in this country or a NAFTA or WTO member country prior to the effective date of the Breen reference. The declaration indicates that at the time the invention was made Anna Sylvan was employed by Pyrosequencing AB and continues to be employed by Pyrosequencing AB. However, this information fails to establish that the reduction to practice of the invention was in this country or a NAFTA or WTO member country prior to the effective date of the Breen reference.

Also, the declaration provides seven pages of notebook. It is noted that the language of the notebook is not English. The examiner is unable to fully consider the evidence since the information has not been thoroughly explained. Further, the notebooks contain graphs and tables which are not legible. The graphs are very small in size which prevents clear analysis of the information depicted. The MPEP requires that "each exhibit relied upon should be specifically referred to in the affidavit or declaration, in terms of what it is relied upon to show." The MPEP also requires the

examiner to thoroughly review the submitted evidence. Since the evidence has not been thoroughly explained and the notebooks are not in English, the examiner has been unable to evaluate the evidence. A more detailed and in depth explanation of the disclosure in the notebook would be needed to evaluate the declaration as a whole.

Thus for the reasons above and those already of record, the rejection is maintained.

7. Claims 8-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Shuber et al. (US Pat. 6,566,101, May 20, 2003) in view of Germer et al. (Genome Research, Vol. 10, No. 2, pages 258-266, February 23, 2000).

Shuber et al. (herein referred to as Shuber) teaches a method for selective nucleic acid sequence detection in single base primer extension reactions of high sensitivity. The methods are useful in detecting small amounts of mutant nucleic acid in a heterogeneous biological sample (abstract). Shuber teaches that samples may be pooled to determine the number of a nucleic acid in a sample (col. 14, lines 60-68). Shuber teaches that the primer extension to identify a single nucleotide polymorphic variant as the present may be performed on combined samples (col. 33, lines 10-15)(limitations of Claim 1). Shuber teaches labeled dNTPs preferably comprise a detection moiety which facilitates detection of the extended primer (col. 5, lines 10-15)(limitations of Claim 1). Shuber teaches to detecting single nucleotide polymorphisms (SNPs) a primer is designed so that the 3' end of the hybridized primer is immediately upstream of the position that is complementary to the nucleotide position being assayed (col. 6, lines 27-32)(limitations of Claim 1). The primers are preferably

extended with a nucleotide labeled with an impedence bead and the number of impedence beads is counted and the number of labeled primers are then determined from the number of impedence beads (col. 7, lines 27-35). Shuber teaches that genomic nucleic acid samples are isolated from a biological sample (col. 7, lines 4-5). Amplification of the specific regions present in the nucleic acids my be amplified by PCR to provide the advantage of increasing the concentration of specific nucleic acid sequences within the target nucleic acid sequence population (col. 7, lines 5-10). The nucleic acids may be bound to a solid phase support which allows the simultaneous processing and screening of a large number of samples (col. 7, lines 13-17)(limitations of Claim 7). Schuber teaches that the method compares the number of molecules of two nucleic acids that are expected to be present in the sample in equal numbers in normal cells. The comparison is between an amount of a genomic polynucleotide that is known or suspected not to be mutated in cells of the sample and an amount of wild-type genomic polynucleotide segment suspected of being mutated in a subpopulation of cells in the sample (i.e. frequency)(limitations of Claim 1).

Shuber does not specifically teach determining the amount or concentration of nucleic acids in each sample prior to pooling.

Germer et al. (herein referred to as Germer) teaches a method for determining the allele frequency of biallelic polymorphisms in pooled samples. Specifically, Germer teaches a mixture of DNAs pooled from individual sample were subjected to primer pairs (e.g. a primer pair specific to one or the other SNP allelic variant), and detecting the frequency (page 259, col. 1). Germer teaches enhancing specificity of the kinetic

PCR reaction by using Stoffel fragment Taq DNA polymerase (page 259, col. 1)(limitations of Claim 3). Germer teaches the amplification efficiencies for the two allele-specific PCRs may differ slightly but this can be measured and compensated for by performing the assay on a DNA known to be heterozygous for the SNP of interest. Germer teaches that the deltaC for this DNA should equal zero if the PCRs are equally efficient (page 259, col. 2). Germer teaches that any deviation can then be subtracted from all deltaC measurements to compensate for differential amplification efficiencies (page 259, col. 2). Germer teaches that error introduced by unequal amplification efficiency of the two allele-specific primers for each polymorphism may be corrected for (page 261, col. 1). Germer teaches method for avoiding the formation and potential interference of template independent generation of primer artifact by using UNG and heat-activated polymerase enzyme. Moreover, using Stoffel fragment of Taq polymerase minimizes the problem because it is highly discriminatory and not very processive (page 260, col. 2). The relative amounts of each allele in a sample are quantified (abstract). As seen in Table 2, the allele frequency measurements on a pool of 100 human DNAs in three genes illustrate very highly correlated results. Germer teaches "in conducting association studies using pools of DNA, accurate quantitation of the individual DNAs is important lest artifactual allele discrepancies between pools arise (page 263, col. 2). Germer teaches that the "simplest safeguard against errors arising from the pooling process would be to validate the pools by doing genotyping of the individual sample and showing concordance between allele counting and frequency measurement on the pool. Germer teaches that Tm-shift genotyping is a good choice

because it uses the same allele-specific PCR conditions and two of the same three primers as the described method (page 263, col. 2). The methods section teaches that the samples were constructed by mixing two homozygous human DNA samples in various proportions by combining known amounts of homozygous DNA samples (limitations of Claim 8). Germer teaches his method of determining SNP allele frequencies in pooled sample has a number of advantages (1) it is not based on expensive fluorescently labeled primers or probes (2) it is a homogenous assay that requires no post-PCR processing (3) it operates under uniform conditions without the need for marker specific assay optimization (4) it is accurate and (5) it promises to be inexpensive, time-saving and precise to allow detection of relatively weak genetic associations (Page 258-259).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the method of Shuber with the specific teachings of Germer that concentrations of samples should be ensured. Germer specifically teaches using the same concentration of each DNA sample is important lest artifactual allele discrepancies between pools arise, however, Germer does not specifically adjusting the amount of nucleic acids to contain substantially the same amount.

However, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the method of Germer which detects concentrations of each DNA sample using a Tm-shift genotyping assay to detect quantities with a further method step of adjusting the concentration. Since Germer

teaches "in conducting association studies using pools of DNA, accurate quantitation of the individual DNAs is important lest artifactual allele discrepancies between pools arise (page 263, col. 2) and the "simplest safeguard against errors arising from the pooling process would be to validate the pools by doing genotyping of the individual sample and showing concordance between allele counting and frequency measurement on the pool", it would have been obvious to adjust the amount or concentration of the nucleic acids present in the event that a discrepancy was ascertained. To obtain accurate information regarding the true frequencies of a pooled population, it is essential that each member of the population is represented in equal concentrations within the sample. For example if the population is comprised of two homozygote individuals, aa and AA, and the pool is designed to contain 10 µg of DNA from the individual with "aa" and 90 µg of DNA from the individual with "AA", the estimated frequency of the alleles would be 0.1 for "a" and 0.9 for "A". This would not accurately reflect the true population frequency. In contrast, a pool designed to contain 50 µg of DNA from the individual with "aa" and 50 µg of DNA from the individual with "AA", the estimated frequency of the alleles would be 0.5 for "a" and 0.5 for "A", a true estimate of the frequencies of the two alleles in the population. Therefore, in order to obtain a true estimate of the frequency of alleles in a population, adjusting the amount or concentration of a nucleic acid in a sample is essential. It would have been obvious to the ordinary artisan to dilute or increase nucleic acid concentrations to ensure equal concentrations within each of the samples prior to pooling.

With respect to Claim 11, Germer teaches assaying for three SNPs in the samples. Therefore, any one of these polymorphisms may be considered a reference polymorphism.

With respect to Claim 12, Germer teaches methods of ensuring that the error introduced by unequal amplification efficiency of the two primers for each polymorphism is corrected. Germer teaches correcting for the error between unequal amplification efficiency. Therefore, it would have been obvious to the ordinary artisan to correct for the polymorphism with respect to background signals and unequal amplification efficiencies.

With respect to Claim 13, the polymorphisms in the PON, B71 and CSTS genes are not located within a homopoymeric sequence, as exemplified by the sequence of the primers provided. Therefore, Germer teaches using a polymorphism no present in a homopolymeric sequence.

With respect to Claim 14-15, Germer teaches DNA from different homozygotes was mixed together in different ratios and analyzed (page 264, col. 2). Table 1 illustrates pooling of samples at different concentrations to determine the relative concentration in each reference sample, thereby generating a calibration cure to correct for the distorted allele frequencies derived from pooling analysis.

Response to Arguments

The response traverses the rejection. The response asserts that Germer only shows whether the method as a whole gives accurate enough measurement on the pool. It does not show how an individual sample deviates or which samples will require

adjustment prior to pooling. This argument has been reviewed but is not convincing because, as stated in the rejection above, to obtain accurate information regarding the true frequencies of a pooled population, it is essential that each member of the population is represented in equal concentrations within the sample. For example if the population is comprised of two homozygote individuals, aa and AA, and the pool is designed to contain 10 µg of DNA from the individual with "aa" and 90 µg of DNA from the individual with "AA", the estimated frequency of the alleles would be 0.1 for "a" and 0.9 for "A". This would not accurately reflect the true population frequency. In contrast, a pool designed to contain 50 µg of DNA from the individual with "aa" and 50 µg of DNA from the individual with "AA", the estimated frequency of the alleles would be 0.5 for "a" and 0.5 for "A", a true estimate of the frequencies of the two alleles in the population. Therefore, in order to obtain a true estimate of the frequency of alleles in a population, adjusting the amount or concentration of a nucleic acid in a sample is essential. It would have been obvious to the ordinary artisan to dilute or increase nucleic acid concentrations to ensure equal concentrations within each of the samples prior to pooling. Germer specifically teaches that samples were individually genotyped for the SNPs and monitored. Thus, Germer teaches determining concentrations of the individual samples prior to pooling.

The response states that Germer does not provide teachings to serve to construct a calibration curve and cannot be used for DNA calibration, as it does not provide information on individual samples. This argument has been thoroughly

reviewed, but is not found persuasive because the claims do not appear to claim a calibration curve based upon individual samples as argued.

Thus for the reasons above and those already of record, the rejection is maintained.

Conclusion

8. No claims allowable over the art.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Goldberg whose telephone number is (571) 272-0743. The examiner can normally be reached Monday-Friday from 7:00 a.m. to 4:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (571) 272- 0745.

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Jeanine Goldberg
Patent Examiner
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